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Bioremediation of Polluted Zanzibar Seawater: The Nutrient and Bacterial Bioextraction Potential of Native Seaweeds and Bivalves

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SIT Spring 2014 Zanzibar: Coastal Ecology and Natural Resource Management
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Acknowledgements

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Abstract

In Stone Town, Zanzibar, increasing populations and insufficient sewage treatment has greatly increased harmful levels of nutrients and anthropogenic-sourced contaminants along the city's coastal waters. In this study, the nutrient and bacterial bioextractive abilities of local species of bivalves (*A. antiquata, P. margaritifera*) and seaweeds (*E. denticulatum*, *U. reticulata*) were examined in order to determine the potential for these species to remedy the local polluted waters. It was hypothesized that due to bivalves' suspension-feeding activity, both species of bivalves would be able to decrease turbidity and fecal indicator bacteria (*Enterococci)* levels in sample polluted seawater. Likewise, it was hypothesized that due to the nitrogen and phosphorous nutrient assimilation of seaweeds, both species of seaweed would be able to decrease excess nutrient levels in the water caused by both raw sewage and bivalve waste. In concordance with extensive aquaculture studies of these effects, both species of bivalves were able to decrease total suspended solids and *Enterococci* levels while both species of seaweeds were able to decrease certain nutrient levels. From this broad study, it was determined that *A. antiquata, P. margaritifera*, *E. denticulatum*, and *U. reticulata* have the ability to be implemented successfully as potential bioremediators in an integrated mariculture program in Stone Town that will prove economically as well as environmentally beneficial.

I. Introduction

Coastal areas around the world are suffering from destruction of coral reefs, unsafe waters for recreational use, and increasing outbreaks of red tides and shellfish poisonings, all of which can be partially accredited to anthropogenic sources of pollution. Stone Town, the main city on the island of Unguja, Zanzibar, is amongst these suffering areas. Due to the absence of a proper sewage system, the waters surrounding Stone Town are being increasingly contaminated through direct flow of raw sewage pipelines and wastewater runoff along the coastline. The concern is not only for the negative impact of such events on tourism, which is economically vital for Zanzibar, but there is also concern for the effect of the pollution on the marine life surrounding the town upon which residents are dependent on as a food source. Due to general lack of funding for improvement of the current sewage system, other methods of alleviation must be evaluated. In other parts of the world, the bioremediation potential of certain marine organisms are being evaluated as a natural and sustainable mechanism for mitigating such effluents. The purpose of this study was to determine the nutrient and bacterial bioextraction potential of local species of bivalves and seaweeds in a laboratory setting, with the hope of future implementation of such a bioremedial approach in Zanzibari waters.

Understanding weather and current patterns specific to the Tanzanian coastline is vital to comprehending the current pollution problem in Zanzibari waters. The nearby East African Coastal Current (EACC) flows to the north during the majority of the year. However, when strong winds are driven from the Tibetan plateau during the northeast monsoon season, the South Equatorial Counter Current (SECC) is formed and thus the EACC is abated (Nyandwi 2014). Conversely, when the southeast monsoon season occurs, the EACC is intensified. Such changes in the strength of the EACC are connected to changes in upwelling and downwelling of nutrient-

rich waters, with the strengthened northward flow of the EACC causing downwelling along the coasts of Tanzania, southern Kenya, and the Zanzibar archipelago. Thus, low-nutrient waters are brought to these coasts, and as warm water temperatures and low nutrient conditions are ideal settings for coral formation, all of these factors contribute towards the high coral biodiversity in these regions (Nyandwi 2014). In these downwelling zones, the main sources of nitrogen are nitrogen fixation, terrestrial runoff, and upwelling, while the main sources of phosphorous are water column mixing, river discharge, terrestrial runoff, and upwelling (McClanahan 1988). Due to increase in rainfall, and consequently runoff, during the monsoon seasons, there is a large increase in nutrients into the ocean (McClanahan 1988).

The nutrient levels in Stone Town, Zanzibar, are affected by not only increases in rainfall, but also improper sewage disposal. There are 2,289 septic tanks in Stone Town, and 27 sewage outflow pipes which extend 55 meters along the ocean floor from the point of low tide (Moynihan et al, 2012). In Stone Town an average of 2,200 m^3 of liquid waste is discharged daily into the ocean, along with the waste entering the ocean from those who do not use the sewage system. The sewage is treated only through decreasing the Biological Oxygen Demand to 60%, but this method is minimally effective (Moynihan et al, 2012). The original sewage system for the city was expected to effectively carry the polluted water through the Zanzibar Channel to be carried away and diluted by the EACC. However, the main current in the Zanzibar Channel contains two eddies caused by the islands of Bawe and Changuu. These

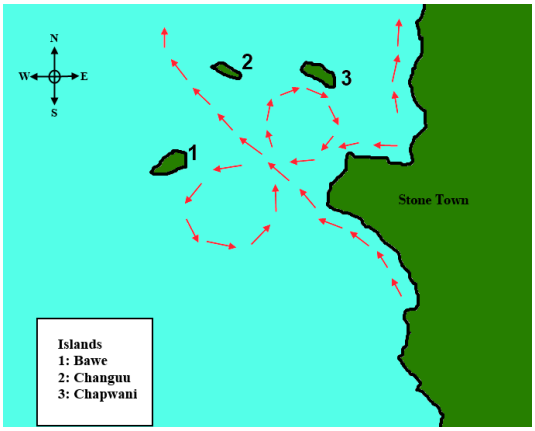


Figure 1: Stone Town and surrounding reef current system (adapted from Anderson 1994).

eddies deter the sewage from properly reaching deeper waters, where it could be mixed with the water column and be diluted to harmless levels (See Figure 1). Thus, either treating the sewage to a greater extent or biofiltering the averted water before the polluted waters accumulate near town is necessary.

Raw sewage is rich in ammonia, organic nitrogen, and phosphate. The problem with sewage-produced nutrient-rich water is an abundance of these nutrients, which are usually limiting growth factors for algae (Grall and Chavaud, 2002). Nitrogen is needed for protein synthesis, and phosphorus is needed for DNA, RNA, and energy transfer (Conley et al., 2009). When excess of these nutrients are available, the algae bloom, creating a mat. The mat then absorbs the sunlight needed for photosynthesis of other aquatic plant life, such as seagrasses. With no oxygen available, the benthic organisms which require oxygen released during photosynthesis—such as bivalves and crustaceans—will die (Grall and Chavaud, 2002). The essential symbiotic zooxanthallae algae on coral is one of these organisms effected (Giollian 2009). Reefs are very sensitive to changes in nutrient concentrations, which decrease coral calcification and fertilization rates (Fabricius 2005) while several coral diseases have been attributed to sewage runoff (Baker et al., 2007).

Furthermore, when raw sewage flows into the ocean, dissolved oxygen is lost first to the biological oxidation of organic carbon to carbon dioxide, and then to the oxidation of ammonia. This lost oxygen is often replenished through the reaeration of the water through turbulence, and also through the photosynthesis of aquatic plants. However, with all of the organic and nitrogen outflow present from sewage, the ability of the receiving water to replenish the diminishing dissolved oxygen is impaired (Pelley 1998). When bacteria use the dissolved oxygen in the water to metabolize accumulating nutrients, the seawater affected become highly anoxic, and promotes

the growth of anaerobic bacteria. The hydrogen sulphide often produced by these bacteria can be toxic to marine life, and the water becomes septic (Pelley 1998). Accumulated sewage in seawater is also detrimental to the health of humans. Algal blooms may render shellfish and fish toxic, or kill them. By 1994, there were 3,164 reported cases of human poisoning and 148 deaths in the Asia-Pacific region alone (Corrales and Maclean 1995). Moreover, sewage runoff is replete with anthropogenic-sourced fecal bacteria. Previous studies have demonstrated that sewage runoff in Stone Town has increased to detrimental levels (Moynihan et al., 2012). Health problems associated with swimming in waters with high levels of fecal bacteria include gastrointestinal illness and respiratory disease (Noble et al., 2003).

One economical and sustainable method for mitigating this increasingly urgent pollution crisis is through bioremediation using marine organisms. Although often demonstrated through an aquaculture system, many different species of bivalves and algae have been shown to have great remediation potential of high-nutrient waters and harmful bacteria (Neori et al., 1998, Chopin et al., 2001, Tyler et al., 2005, Timoney and Abston, 1984). The organisms chosen for this experiment are species native to Zanzibar, and three of the four species are commercially cultivated. The bivalves chosen were *Anadara antiquata* and *Pinctada margaritifera*, both of which are native to the Indo-Pacific. *A. antiquata* (feather cockle) can grow up to 7 cm. wide, are eulittoral and therefore usually bury in muddy sand, and are an important source of food for coastal populations in the region (Richmond 284). *P. margaritifera* (black-lip pearl oyster can grow up to 10 cm., are found on shallow rocks or coral rubble, and sometimes produce grey to black pearls (Richmond 286). Locally, *A. antiquata* are grown as an important food source, while the *P. margaritifera* are cultivated for their beautiful shells and pearls.

Bivalves are suspension-feeding organisms, so they obtain their food from the surrounding water by collecting suspended organic particles when water passes through the gills (Winter 1978). These particles can consist of phytoplankton, suspended silt and clay particles, and detritus particles (Park et al., 2008). These particles are then either passed to the gut and digested or excreted, or immediately rejected as pseudofaeces (Newell and Jordan 1983). Although their food source is microalgal nanoplankton, bivalves are generally indiscriminate filter feeders and therefore filter all suspended matter (Gifford et al., 2005). As a result, it was hypothesized that both *A. antiquata* and *P. margaritifera* would be effective filterers of the high amount of organic suspended particles in the highly turbid water surrounding Stone Town, and also potential bioextracters of the high levels of anthropogenic-sourced bacteria, namely *Enterococci*, found in high levels in Stone Town coastal waters (Moynihan et al., 2012). For example, *Mercenearia mercenaria* (American hard clam) is able to filter $7.5*10^7$ *E. coli* cells, and retain about one tenth of these cells in its tissues after only fifteen minutes of exposure to the sewage (Timoney and Abston, 1984). In this study, bacterial bioextraction ability was determined through *Entercocci* counts, a fecal indicator bacteria which is considered the best indicator for sewage presence in seawater due to its high salinity tolerance (Noble et al., 2003). However, bivalves transform part of the nutrients in consumed microalgae to dissolved and bio-available forms—such as ammonium, urea, and phosphate—making the nutrients available for use by other organisms, such as harmful algae blooms (Newell, 2004). Thus, a secondary biofiltration organism is needed to decrease these nutrient levels. In this study, two species of algae are proposed for this utilization.

The seaweeds chosen were *Ulva reticulata* and *Eucheuma denticulatum*, which are similarly native to the Indo-Pacific. *U. reticulata* (green algae) is dark green seaweed that is

highly perforated and composed of varying-sized stiff blades. As it is often entangles with other algae, it is present from the sublittoral to the high tide level, and is often abundant in the vicinity of large coastal cities as an indication of eutrophication (Richmond 82). *E. denticulatum* (red algae) is a brown and green seaweed with spiny, cylindrical branches that is often attached to corals or in lagoons in the lower eulittoral (Richmond 98). The *E. denticulatum* is grown locally in successful seaweed farming endeavors, while the *U. reticulata* is not grown commercially and is often found on shore at high tide. Marine macroalgae, or seaweeds, have been shown numerous times to effectively strip nutrients from both aquaculture effluents and in natural estuaries in the environment (Chopin et al., 2001). Cultivated seaweeds are a net sink for nitrogen and phosphorus, because they uptake nutrients from the water that are effectively removed from the system when the seaweed is harvested (Chopin et al., 2001). Seaweeds are effective biofilters because of their high growth rates, which requires nitrogen acquisition. In fact, implementing a new area of integrated seaweed farming in the proposed site of implementation may actually help the seaweed farming industry, as seaweeds have higher growth rates in nutrient-rich waters due to no limiting growth factors (Neorit et al., 2000). Although studies have shown *U. reticulata* to have the highest nutrient uptake rate (Myusa et al., 1999, Neori et al., 1998), the harvested biomass has low commercial value (Rodriqueza and Montano 2007). In contrast, *E. denticulatum* is still considered efficient in taking up nutrients rapidly due to their ability to store large reserves of nutrients and the extracted biomass has high commercial value (Rodriqueza and Montano 2007). In studying the individual abilities of *A. antiquata, P. margaritifera*, *E. denticulatum,* and *U. reticulata* to alter turbidity, bacteria, and nutrient levels, the potential for implementing an integrated mariculture system to remedy polluted Stone Town seawater in an economical and sustainable way can be better understood.

II. Study Area

Unguja is one of two main islands in the Zanzibar archipelago, located 35 km east of mainland Tanzania (S 06° 10.0', E 039° 20.0'). The sample water for all experiments was collected from Stone Town, Zanzibar, on the shore outside of the Institute of Marine Science (S 06° 9'28.72" E 39°11'30.77"), Figure 2. The site of collection is adjacent on one side to the Dar Es Salaam ferry port, and on the other side to a boat-repair area where locals frequently swim and small charter boats to nearby reefs depart. The nutrient analysis experiments, filtration, and total suspended solids analyses were carried out at the Institute of Marine Sciences, University of Dar Es Salaam, Zanzibar. The bacterial analysis filtration and incubation were completed at the Zanzibar Water Authority (ZAWA) Laboratory, near Stone Town, Zanzibar.

Figure 2: Unguja, Zanzibar, with site of water collection marked.

III. Methodology

III. A: Nutrient Analysis Experiments

Seawater Collection:

Seawater was collected 20 meters offshore the beach behind the Institute of Marine Sciences, in Stone Town, Zanzibar. Three, 10 L sterilized containers were filled with water 6 inches below the surface, rinsing the container three times with the sample water before collection. Sand was collected approximately 1 meter offshore, and placed in a sterilized bucket (See Appendix 1: Table 1 for date, weather, and tide at time of collection). Each 5in by 10in experiment tank is filled with 2.5 L of sand, and 3 L of seawater.

General Experimental Set-Up:

After each experiment's specific set-up, 3 350mL containers were filled with the seawater to serve as the 'Initial' readings for the experiment. Temperature, pH, and observations were recorded initially and finally and temperature was recorded every 15 minutes for the duration of the experiment. After the experimental run, six 350mL sterilized containers were filled with the 'Final' seawater, one from each experimental and control tank respectively (3 experimental, 3 control). The labeled containers were then frozen in a -20˚C freezer until analyzed (See Appendix 1: Table 2 for the pH, average temperature, and total time of experiment for all experimental set-ups in this study).

Figure 3: Sample standard experimental set-up.

***A. antiquata Experiment*:**

10 *A. antiquata* of comparable size were placed in each of the 3 experimental tanks, while none were added to the control tanks. The *A. antiquata* were given 24 hours to bury themselves into the sand, so all experimental and control tanks were filled with 2.5 L of sand, and placed in a large, aerated holding tank until the experiment. The tanks were removed from the aerated holding tank, and all remaining water was suctioned out. 3 L of the sample seawater was added, and the *A. antiquata* were allowed to acclimate for 30 minutes. The 'general' experimental procedure was then followed.

***P. margaritifera Experiment*:**

20 *P. margaritifera* of comparable size were placed in each of the 3 experimental tanks, while none were added to the control tanks. All experimental and control tanks were filled with 2.5 L of sand, and placed in a large, aerated holding tank until the experiment. The tanks were removed from the aerated holding tank, and all remaining water was suctioned out. 3 L of the sample seawater was added, and the *P. margaritifera* were allowed to acclimate for 30 minutes. The 'general' experimental procedure was then followed.

***E. denticulatum* and *U. reticulata Experiments*:**

For both experiments, approximately 100 grams of the respective seaweed was added to each of the 3 experimental tanks, while none was added to the control tanks. After allowing the seaweed to acclimate for 30 minutes, 3 350mL containers were filled with the seawater to serve as the 'Initial' readings for the experiment. The 'general' experimental procedure was then followed.

***A. antiquata* and *E. denticulatum Experiment*:**

10 *A. antiquata* of comparable size were placed in each of the 3 experimental tanks, while none were added to the control tanks. The *A. antiquata* were given 24 hours to bury themselves into the sand, so all experimental and control tanks were filled with 2.5 L of sand, and placed in a large, aerated holding tank until the experiment. The tanks were removed from the aerated holding tank, and all remaining water was suctioned out. 3 L of the sample seawater was added, along with approximately 100 grams of seaweed, and the organisms were allowed to acclimate for 30 minutes. The 'general' experimental procedure was then followed.

***P. margaritifera* and *U. reticulata Experiment*:**

20 *P. margaritifera* of comparable size were placed in each of the 3 experimental tanks, while none were added to the control tanks. All experimental and control tanks were filled with 2.5 L of sand, and placed in a large, aerated holding tank until the experiment. The tanks were removed from the aerated holding tank, and all remaining water was suctioned out. 2.5L of sand and 3L of the sample seawater was added, along with approximately 100 grams of seaweed, and the organisms were allowed to acclimate for 30 minutes. The 'general' experimental procedure was then followed.

Filtration, Total Suspended Solids, Nutrient Analysis:

After weighing each Glass Fiber Filter paper (Advantec 47mm), each 350mL sample was filtered through a sterilized vacuum filtration device. The filter paper and solid residue were placed in an incubator oven at 60˚C to bake overnight, and the total suspended solid weight was recorded twenty hours later. The filtered water was placed back into its original container, and 10mL samples were pipetted into reaction vials for ammonium and phosphate analysis. 100mL were placed into flasks for nitrate analysis. Each nutrient sample, its necessary reagent, and its

standard calibration curve were prepared according to standard methods in the Intergovernmental Oceanographic Commission Manual (1993). See Appendix 3 for standard calibration curve details.

Ammonium: To each 10mL reaction vial, 1mL of a 1 : 2.5 : 1 mixture of phenol solution: alkaline solution/hypochlorite (4:1) : sodium nitroprusside was added. The samples were then incubated at room temperature in a dark drawer overnight, and the absorbance was measured and recorded at 640nm using a UV Spectrophotometer.

Phosphate: After initial filtration, the 10mL reaction vials were frozen overnight. After thawing, to each reaction vial 1mL of a 1 : 2.5 : 1 : .5 mixture of ammonium molybdate: sulfuric acid: ascorbic acid: potassium antimonly-tartrate was added. The samples were then incubated at room temperature for two hours. The absorbance was measured and recorded at 885nm using a UV Spectrophotometer.

Nitrate: 2mL of analytical grade ammonium chloride solution (125g NH_4Cl : 500mL distilled water) was added to 100mL of each filtered sample. Each 100mL was then passed through a cadmium-copper column at a rate of 10 minutes per 100mL, with the first 50mL discarded. To the remaining collected 50mL, 1mL of sulfanilamide reagent and 1mL of N-(1-naphthyl)-ethylenediamine dihydrochloride reagent were added. The absorbance was measured and recorded at 543nm using a UV Spectrophotometer.

III. B: Bacterial Analysis Experiments

Seawater Collection:

Seawater was collected with the same methodology as described previously for 'Nutrient Analysis Experiments'.

Experimental Set-Up:

All experimental set-ups for each respective organism—*A. antiquata*, *P. margaritifera*, and *E. denticulatum*—are identical to those described for 'Nutrient Analysis Experiments', with the exception that each experiment lasted two hours, and the collected water was immediately transported on ice to ZAWA instead of refrigeration for later analysis.

Bacterial Filtration and Incubation Protocol:

Enterococci were enumerated using membrane filtration (USEPA Method 1600, 2002). Each sample was diluted to $1/8^{th}$, thus 12.5mL of sample was added to 87.5mL of distilled water. The diluted sample was then passed through a sterilized vacuum filtration device onto a membrane filter (GelmanSciences Sterilized Membrane). The membrane filter was removed and placed on a labeled petri dish prepared with growth agar (see Appendix 3 for agar preparation). After all 12 samples were diluted, filtered, and placed onto the agar, the petri dishes were placed in a 37˚C incubator for 48 hours. After 48 hours, the petri dishes were removed and all colonies were counted and normalized to colony forming units per 100mL ($CFUx100mL^{-1}$)**

$$**CFUx\ 100mL^{-1} = \frac{\text{original colony count}}{\text{mL of sample filtered}} \text{ x } 100$$

IV. Results

IV. A: Total Suspended Solids

For both *A. antiquata* and *P. margaritifera*, the total suspended solids in the collected experimental water was significantly less as compared to the control (t= 2.7764, p= .0372, and t= 2.7764, p=.0297 respectively). Conversely, for both of the seaweeds, *E. denticulatum* and *U. reticulata,* the total suspended solids in the collected experimental water was significantly more as compared to the control (t= 2.7764, p= .0486, and t= 2.7764, p= .7225, respectively). For the integrated experiments, the total suspended solids in the collected water for *A. antiquata* and *E. denticulatum* was significantly less as compared to the control (t= 2.7764, p=. 0254), while there was no significant difference between the experimental and control with *P. margaritifera* and *U. reticulata* (t= 2.7764, p=.1534). See Figure 1 for *U. reticulata* and *P. margaritifera* results, and Figure 2 for *E. denticulatum* and *A. antiquata* results. (See Appendix 1: Table 3 for enumerated table of results).

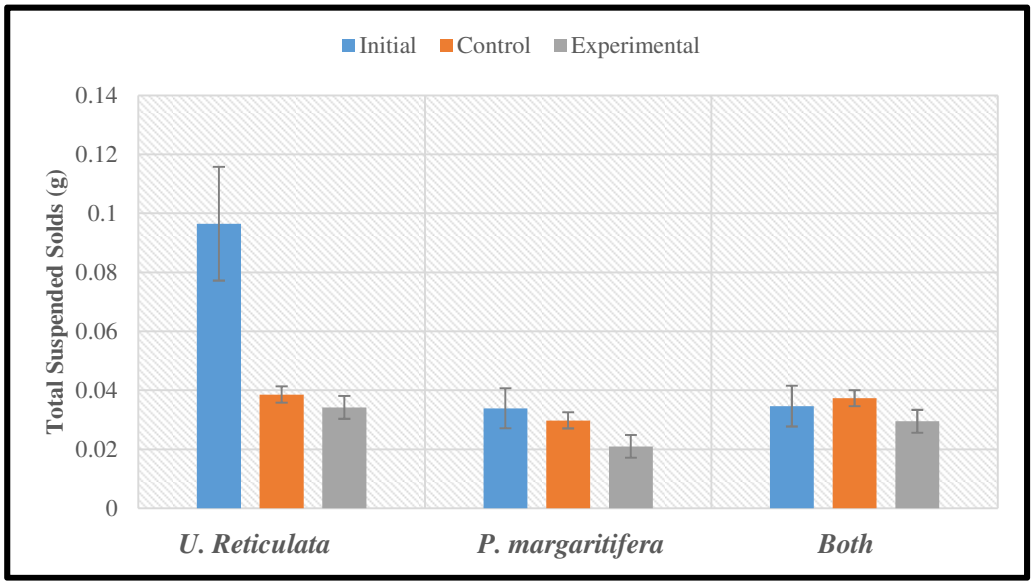


Figure 4: The average total suspended solids per 350mL of collected water for *U. reticulata*, *P. margaritifera*, and the combination of each. T-tests results showed a significant difference between the control and the *P. margaritifera* sample, between the control and the *U. reticulata* sample, but no significant difference the combination of each. Error bars represent one +/- standard deviation.

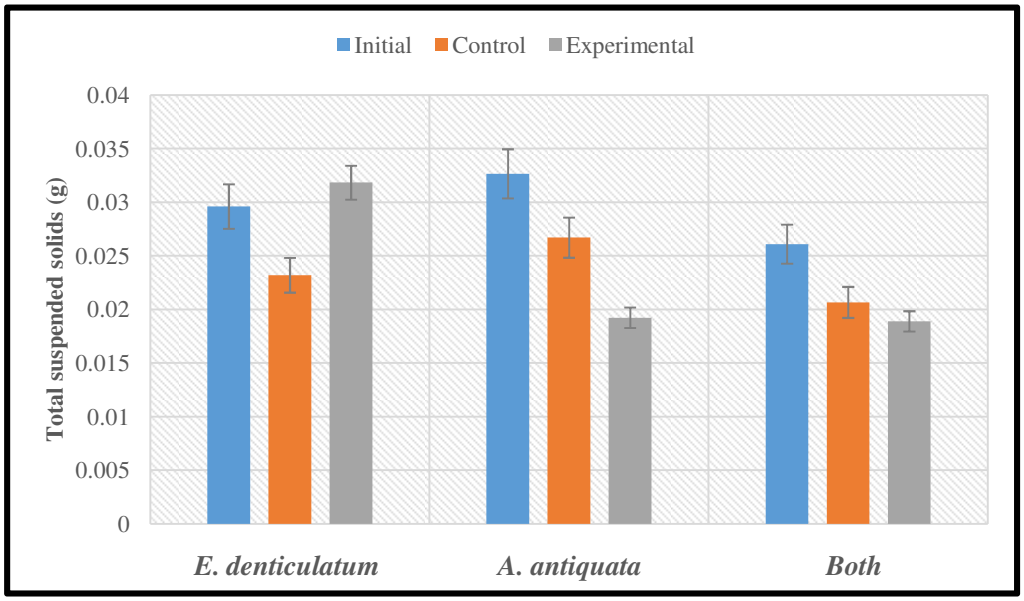


Figure 5: The average total suspended solids per 350mL of collected water for *E. denticulatum*, *A. antiquata*, and the combination of each. T-tests results showed a significant difference between the control and each experimental sample. Error bars represent one +/- standard deviation.

IV. B: Bacterial Analysis

For both the *P. margaritifera* and *A. antiquata* experiments, the total colony count of *Enterocci* was significantly less as compared to the control (t=2.7764, p=.0051, and t=2.7764, p=.0157 respectively). However, there was no difference between the total colony count of the *E. denticulatum* experiment and the control (t= 2.7764, p=.1831). See Table 1 for the total colony counts (CFUx100 mL^{-1}), average, and standard deviation for each experiment, and Figure 5 for visual results.

	Sample #1	Sample #2	Sample #3	Average	Standard Deviation
Control	2488	2128	1632	2082.667	±53.725
P. margaritifera	744	688	672	701.333	±4.726
E. denticulatum	1928	1544	912	1461.333	±64.128
A. antiquata	1200	936	912	1016.000	±19.975

Table 1: The total colony counts (CFUx100 mL^{-1}) of each sample for the control, *P. margaritifera*, *E. denticulatum*, and *A. antiquata* bacteria experiments.

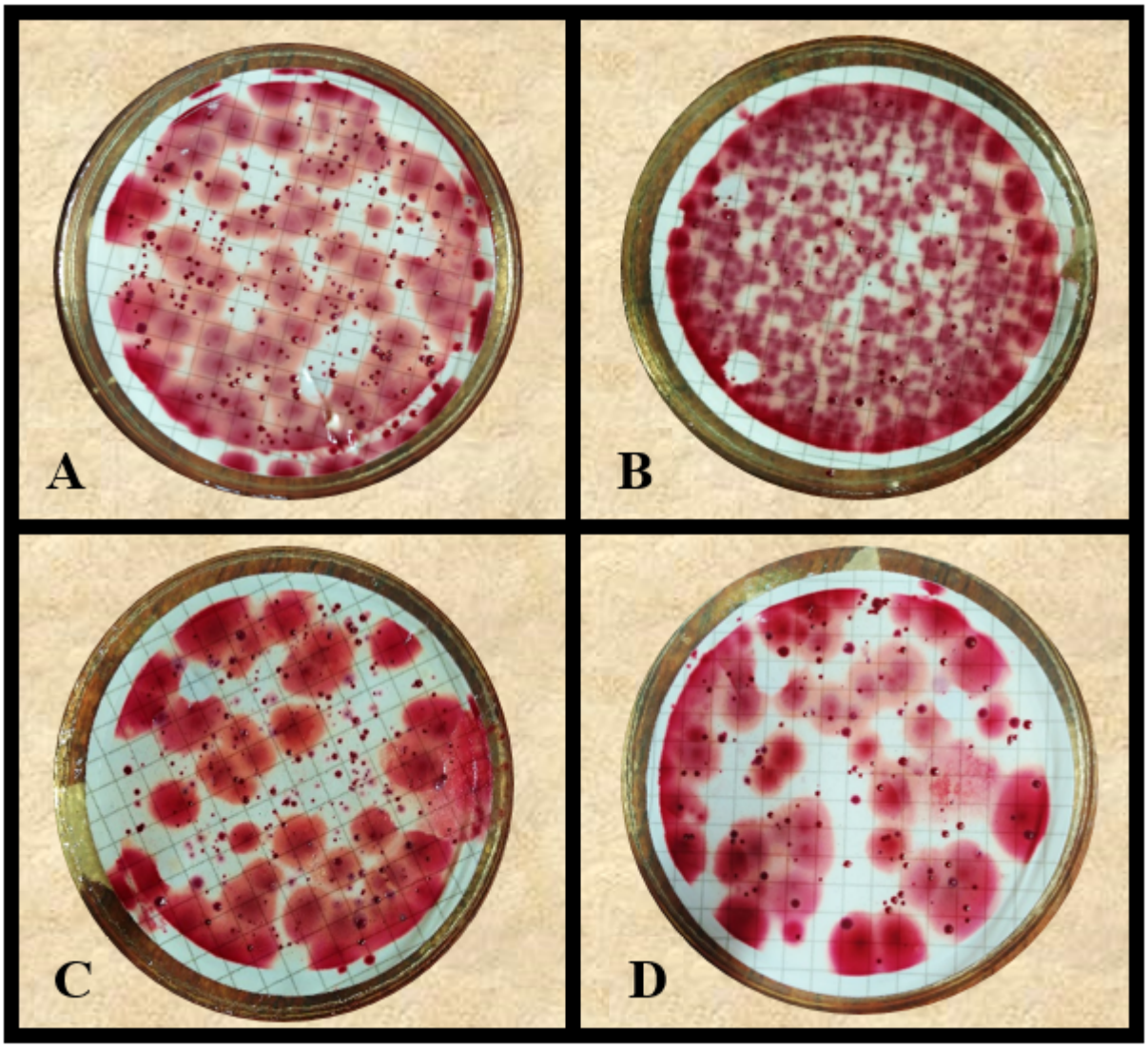
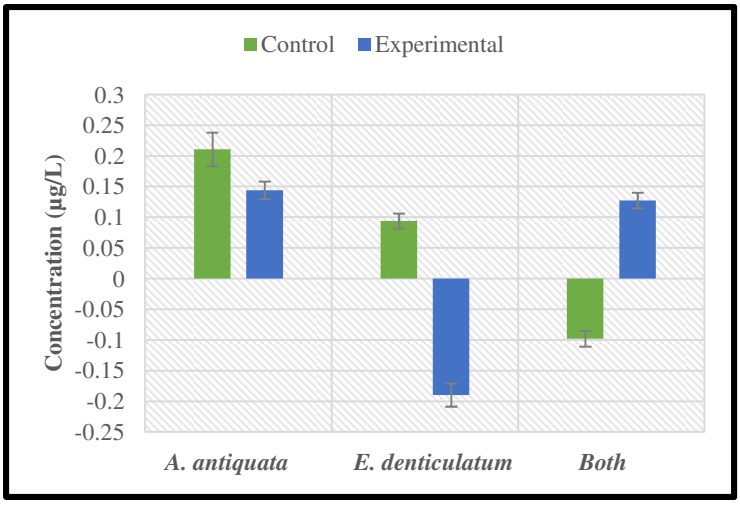


Figure 6: The images above are the results of the bacterial analysis. The small, shiny red circles were counted as colonies. The blurry, larger patches are a result of air bubbles during application and are not any indication of colonies. Image A) is the control, with a total of 266 colonies. Image B) is the result of the *P. margaritifera* experiment, with a total of 84 colonies. Image C) is the result of the *E. denticulatum* experiment, with a total of 193 colonies. Image D) is the result of the *A. antiquata* experiment, with a total of 140 colonies.

IV. C: Nutrient Analysis

Phosphate:

Although the *A. antiquata* decreased overall phosphate concentration, there was no significant difference between the experimental and the control (t= 2.7764, p= .09444). The *E. denticulatum* significantly decreased the phosphate levels as compared to the control (t= 2.776, p=.05333). The combination of both *A. antiquata* and *E. denticulatum* caused a significantly increased concentration of phosphate (t= 2.7764, p= .0226). The *P. margaritifera* significantly increased the phosphate concentrations (t= 2.7764, p= .0001), while the *U. reticulata* decreased the phosphate concentrations but not significantly (t= 2.7764, p= .1120). The combination of both *P. margaritifera* and *U. reticulata* caused a non-significant decrease in the concentrations (t=3.18244, p= .2339). See Figure 6 for a graphical representation.



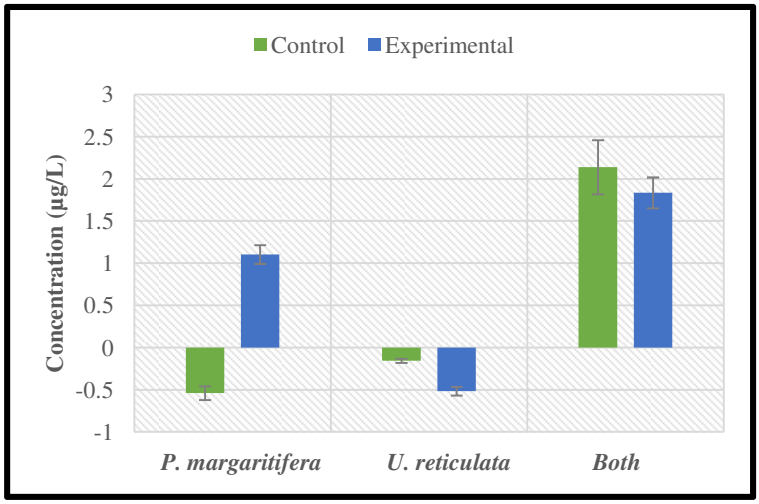
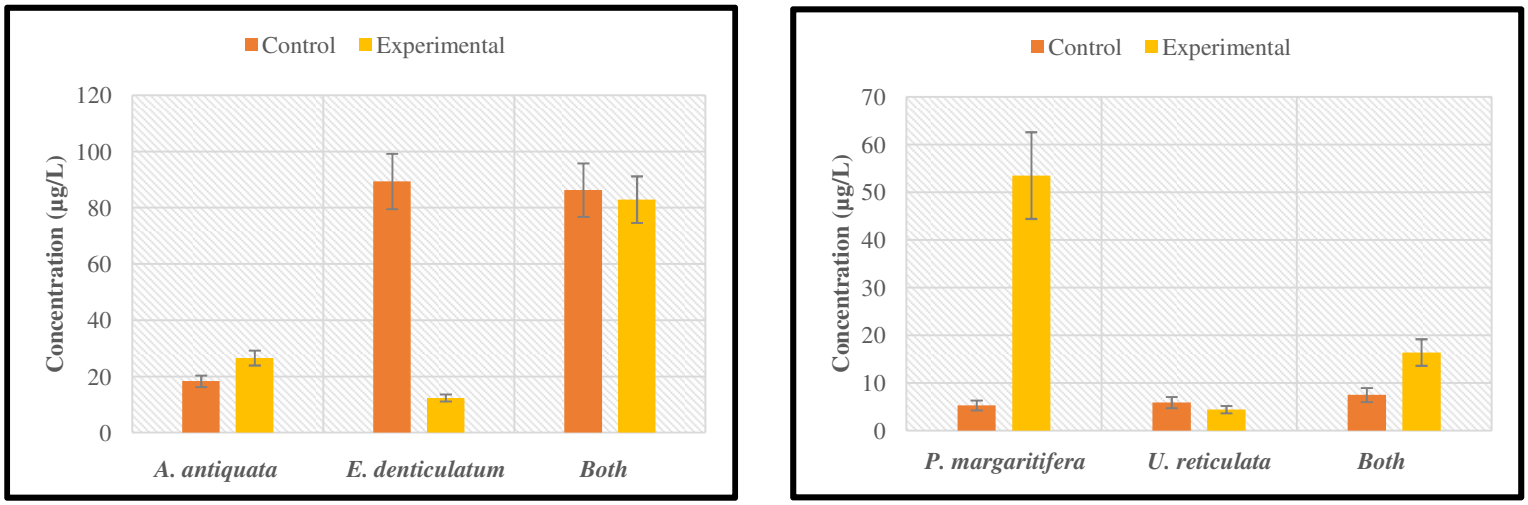


Figure 7: The average phosphate concentration (µg./L) for all experiments. T-tests results showed a significant difference between the control and *E. denticulatum*, *A. antiquata* + *E. denticulatum*, and *P. margaritifera*. Error bars represent one +/- standard deviation.

Ammonium:

The *A. antiquata* increased the overall ammonium concentration significantly (t= 2.1132, p= .0042). The *E. denticulatum* significantly decreased the ammonium levels as compared to the control (t= 2.1318, p < .0001). The combination of both *A. antiquata* and *E. denticulatum* caused a decrease in the concentration of ammonium (t= 2.1318, p= .3644). The *P. margaritifera* significantly increased the ammonium concentrations (t= 2.7764, p= .0034), while the *U. reticulata* significantly decreased the ammonium concentrations (t= 2.7764, p= .0344). The combination of both *P. margaritifera* and *U. reticulata* caused significant increase in the concentrations (t=2.7765, p < .0001). See Figure 7 for graphical representation.

Figure 8: The average ammonium concentration (µg./L) for all experiments. T-tests results showed a significant difference between the control and *A. antiquata*, *E. denticulatum*, *P. margaritifera*, *U. reticulata*, and *P. margaritifera* + *U. reticulata*. Error bars represent one +/- standard deviation.



Nitrate:

The *A. antiquata* increased the overall nitrate concentration significantly (t= 4.3027, p= .0172). The *E. denticulatum* decreased the nitrate levels, but not significantly (t= 4.3027, p= .0897). The combination of both *A. antiquata* and *E. denticulatum* caused a significant decrease in the concentration of nitrate (t= 4.3027, p= ..0172). The *P. margaritifera* decreased the nitrate concentrations (t= 4.3027, p= .08354), while the *U. reticulata* significantly decreased the nitrate concentrations (t= 4.3027, p= .0032). The combination of both *P. margaritifera* and *U. reticulata* caused significant decrease in the concentration (t=4.3027, p= .0475). See Figure 8 for a graphical representation.

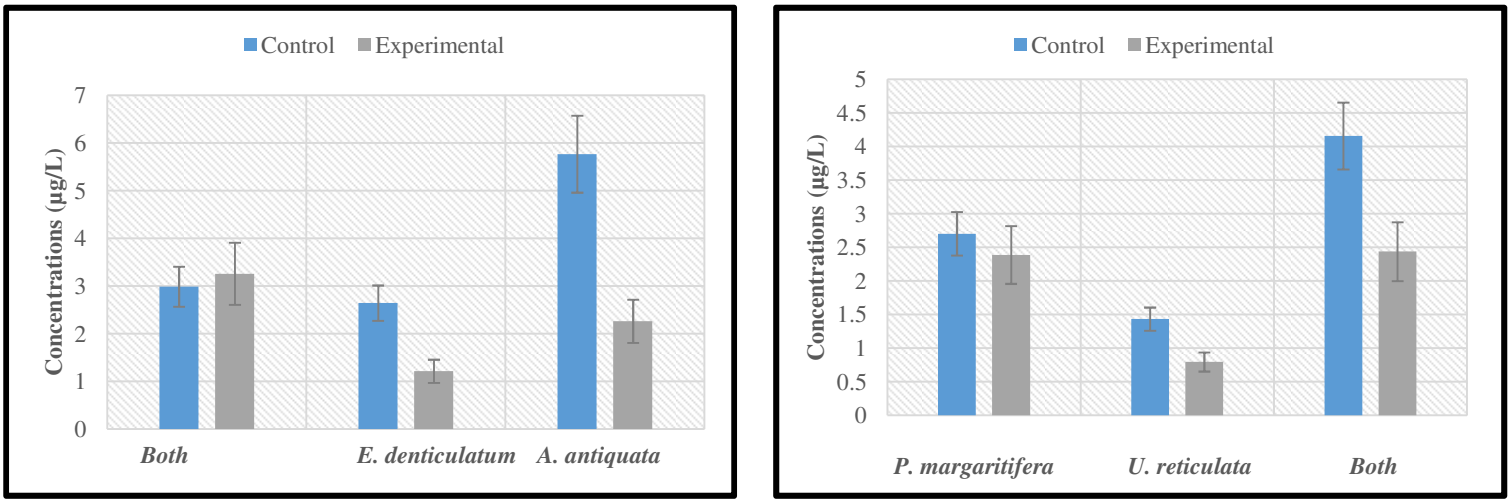


Figure 9: The average nitrate concentration (µg./L) for all experiments. T-tests results showed a significant difference between the control and *A. antiquata*, *E. denticulatum* + *A. antiquata*, *U. reticulata*, and *P. margaritifera* + *U. reticulata*. Error bars represent one +/- standard deviation.

V. Discussion

The current practice of releasing sewage effluents into the water surrounding Stone Town, and the insufficient dilution of this sewage through inadequate current levels and eddies created by the islands of Bawe and Changuu, is increasing biotoxins due to the mixing of seawater and sewage. These biotoxins include nitrates, phosphate, ammonium, and various bacteria and microorganisms. With high concentrations of these nutrients, phytoplankton, zooplankton, and bacteria become over-populated and thus increase the turbidity and virulence of the seawater and thus contribute to the collapse of local ecosystems. Bivalves and seaweed have all been experimentally proven to decrease certain bacteria and also eutrophication levels in the water, thus removing nitrogen and decreasing turbidity while decreasing anthropogenic-sourced bacteria. Given the importance of Stone Town's surrounding reefs for both tourism and as a food source, and also on the importance of the health concerns of swimming in polluted water, an economically feasible and effective solution such as an integrated mariculture approach is necessary.

Total Suspended Solids:

Both bivalves were able to significantly decrease total suspended solids from their surrounding water column. However, the sample water collected from the experimental tanks of both species of seaweed demonstrated overall higher levels of total suspended solids than the control water. Although all seaweed was carefully rinsed before the experiment, it was observed as the experiment period continued that small pieces of seaweed were loosened and floated in the experimental water, thus increasing the visible turbidity. As such, it is understandable that only one of the experimental tanks integrating both a bivalve and seaweed demonstrated lower levels

of total suspended solids as compared to the control. The seaweed was shown to increase turbidity, and the bivalves to decrease it, so the net total was not significantly different from the control. This decrease in turbidity is an important bioremediation mechanism in order to allow for greater sunlight penetration to keystone photosynthesizing organisms, like the zooxanthallae algae of coral. Thus, the integrated organisms studied will be able to effectively contribute towards decreasing suspended matter in the water column in the highly turbid waters surrounding Stone Town.

Enterococci Analysis:

Supporting the hypothesis, the sample water collected from both *A. antiquate* and *P. margaritifera* experimental tanks had resulting *Enterococci* colony counts that were significantly less (1016.00 and 701.33 CFU x $100mL^{-1}$) than the control sample water (2082.667 CFU x $100mL^{-1}$). Conversely, *E. dentituclatum* did not demonstrate bioextractive abilities (1461.33 CFU x $100mL^{-1}$). *Enterococci* are a fecal indicator bacteria which is considered the best indicator for sewage presence in seawater due to its high salinity tolerance (Noble et al., 2003). Although the fecal indicator bacteria are not necessarily harmful in themselves, they represent anthropogenic-sourced bacteria and these results suggest that these bivalves are likely able to filter some of the more harmful microorganisms which are also found in human sewage due to their indiscriminate filtering ability (Gifford et al., 2005). However, even the bivalve-remedied water has a count significantly above 104 CFU x $100mL^{-1}$, which is the level that USEPA recommends for safe swimming waters. (United States Environmental Protection Agency, 1986). Thus, this study also reveals that the levels of harmful microorganisms in Stone Town's waters are substantially higher than recommended, and further denotes the importance of creating a solution to this problem.

Nutrient Analysis:

Although some nutrient assimilation trends were analogous between species, the overall results of the nutrient analysis were varying. *A. antiquata* increased ammonium (control= 18.3111 µg/L, experimental= 26.6012 µg/L) and nitrate (control= 2.9860 µg/L, experimental= 3.2564 µg/L), while the oysters increased phosphate (control= -.5402 µg/L, experimental= 1.1033 µg/L) and ammonium (control= 5.2813 µg/L, experimental= 53.5127 µg/L). *E. denticulatum* decreased phosphate (control= .0938 µg/L, experimental= -.1898 µg/L) and ammonium (control= 89.3186 µg/L, experimental= 12.3700 µg/L), while *U. reticulata* decreased ammonium (control= 5.8985 µg/L, experimental= 4.4108 µg/L) and nitrate (control= 1.4313 µg/L, experimental= .7932 µg/L). So in general, the bivalves increased nutrients while the seaweeds decreased nutrients. The results of the combined bivalves and seaweeds had varying results: the *A. antiquata* and *E. denticulatum* increased phosphate (control= -.0980 µg/L, experimental= .1272 µg/L) but decreased overall nitrate (control= 5.7664 µg/L, experimental= 2.2595 µg/L), while the *P. margaritifera* and *U. reticulata* increased the ammonium (control= 7.4710 µg/L, experimental= 16.3907 µg/L) but also decreased overall nitrate (control= 5.7664 µg/L, experimental= 2.2595 µg/L). As there is a lot of information for each species, see Table 1 for a simple summary of all significant results.

Table 1: Basic summary of all significant results for each experimental species.

With these preliminary results, it is proposed that the oysters and cockles can be used for biofiltration of harmful bacteria and suspended organic matter, while the seaweed can serve as a secondary biofiltration system for the nutrients. As shown by the increases in ammonium, nitrate, and phosphate, bivalves transform part of the nutrients in consumed microalgae to dissolved and bio-available forms, making the nutrients available for use by other organisms (Newell 2004). Conversely, the seaweeds proved to assimilate phosphate, ammonium, and nitrate, with the nitrogen used for protein synthesis, and the phosphorus needed for DNA, RNA, and energy transfer (Conley et al., 2009). Due to limitations of resources, the ratio of bivalves to seaweed in the combinatorial experiments was likely not ideal: Larger amounts of seaweed biomass may be needed to effectively decrease excess nutrients. However, at the large scale of the proposed implementation, seaweed biomass will be drastically increased and therefore more effective.

Implementation Logistics:

As this study focused on the bioextractive abilities of native species of bivalves and seaweed in a laboratory setting, actual cultivation practices and implementation logistics were not studied. However, a comprehensive study by Oakland in 2013 assessed the current cultivation practices of oysters and sponges in Zanzibar waters, and determined that, if implemented, such an integrated mariculture program would be straightforward, economically beneficial, and sustainable. Due to potentially high levels of harmful bioaccumulators present, it is presented

Organism	**Significant Changes (p= < .05)**
A. antiquata	Decreased turbidity, decreased bacteria, increased ammonium/nitrate
P. margaritifera	Decreased turbidity, decreased bacteria, increased phosphate/ammonium
E. denticulatum	Increased turbidity, decreased phosphate/ammonium
U. reticulata	Decreased ammonium/nitrate
A. antiquata + *E. denticulatum*	Decreased turbidity, increased phosphate, decreased nitrate
P. margaritifera + *U. reticulata*	Increased ammonium, decreased nitrate

that the feather cockles, if used, can be sold as fertilizer rather than as a food source. Likewise, the pearl oysters shells and pearls can still be used for revenue, while the removed meat can be sold as fertilizer. The red algae can still be exported for use in the pharmaceutical industry. With regular maintenance and surveillance, the sustainable practices of integrated bivalve and seaweed mariculture will ensure that excess numbers of these organisms, leading to an ecological imbalance, will not occur. Thus, the implementation of an integrated mariculture bioremediation farm will not only improving local water quality for the benefit of marine and human life but also contributing towards a profitable local industry. It has been suggested that a potential site for such a bioremediation implementation is at the coast along Maruhubi Ruins (Oakland 2013). Due to the current system along the western coast of Unguja, a large accumulation of bacteria and nutrients is found here. In fact, it has been shown that ammonium and bacteria levels are higher in this area than the waters immediately surrounding Stone Town (Moynihan et al., 2012). As the site of a local fishing village, there is no tourist presence on the beaches, and the shallow depth of the water allows for probable successful implementation of bivalve and seaweed cultivation. Furthermore, the polluted seawater averted from Stone Town would pass through these bioremediation waters before reaching fringing reefs or the feedback water circulation system (Oakland 2013). Further assessing of the site and involvement of the Department of the Environment is necessary to begin the process towards implementing this bioremediation strategy.

Limitations

Due to the short time period for this experiment, there were several errors which occurred that may have skewed results. This study was done in a static experimental tank, with no water flow. The filtering abilities of the bivalves are dependent on water flow that delivers particles to their

siphons. Therefore, results in the field, if implemented, will likely be significantly different than those observed in this study. Although it was demonstrated that the *Enterococci* levels in the water column decreased significantly more with the bivalves, only the water column was tested and therefore the bacteria could be present in the bivalves' feces, thus creating the possibility that the bacteria was in fact not integrated. Furthermore, it is recommended to immediately filter and test the nutrient levels of seawater, especially the ammonium levels. However, due to lack of resources and time, the samples had to be frozen until analysis, which may have skewed results. Longer periods of experimentation and sample collection may be necessary in order to gain a greater understanding of the cycling of nutrients and true assimilation of such nutrients by the organisms studied.

VI. Conclusion

A. antiquata, P. margaritifera, E. denticulatum, and *U. reticulata* have demonstrated specific abilities to remedy certain aspects of unfavorable seawater conditions increased through anthropogenic-sourced waste in Stone Town, Zanzibar. Both bivalves were able to decrease *Enterococci* and total suspended solid levels, while both seaweeds were able to decrease nitrogen and phosphorous levels. Implementing a bioremediation program utilizing these organisms will be a revenue-generating option to the concerning pollution levels surrounding the city. With little to no funding for sewage treatment improvements, designing a sustainable system for pollution removal from seawater while actually increasing profit is a great opportunity. As both species of bivalves and *E. denticulatum* are cultivated commercially in Zanzibar, the resources and knowledge already exist for successful future execution. With the extreme reliance Zanzibar holds on unpolluted coastal waters— for safe swimming waters, pristine coral reefs, and nonhazardous seafood consumption—steps need to be taken to begin to remedy the concerning amount of sewage accumulation. The proposed use of the organisms researched in this study has the potential to provide such a measure of successful future remediation.

VII. Recommendations

As this was a very broad and preliminary analysis, there is an abundance of research that needs to be completed before implementation of proposed bioremediation methods. Repeating these nutrient analysis experiments at different time intervals may give a clearer picture of the underlying mechanisms of nutrient equilibrium and cycling within the integrated system, as the duration of the experiments in this study were brief. For bivalves, age of greatest filtration abilities should be determined, in order to ascertain the most productive timeframe in the bivalves' lifespan which can be correlated with collection timetables. Furthermore, determining individual species' abilities in correlation with pollution levels will be vital in order to establish an optimal location of implementation. May different environmental and biological factors affect the ability of bivalve filtration, such as rainfall, tidal cycle, and wind direction (Lee et al., 2003). Likewise, nutrient uptake by seaweed depends greatly on climate, season, temperature, and many other factors (Tyler et al., 2005). Thus, research into the effects of any of these factors on the assimilation abilities of the species in this study will be highly beneficial. The abilities of the different species were not able to be compared in this study as the water collected, and consequently water quality conditions, were not controlled between the different experimental days. Thus, further studies done on the actual comparison of the two species would be beneficial in determining which organisms would be most effective in implementation. Determining the assimilated nutrient and bacteria levels of utilized bivalves is vital for not only a clearer understanding of actual assimilation rates, but also as a study for potential use as a fertilizer. Research into the logistics of selling collected bivalve flesh as fertilizer to local farmsteads should be evaluated as an effective means of contamination removal. Finally, a comprehensive evaluation of the logistics of bioremediation mariculture at the proposed site of location is essential in order to begin the preliminary phases of operation.

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IX. Appendices

***Appendix I**: Miscellaneous Tables*

Experiment	Date	Time	Tide	Weather (past 24 hours)
Nutrient Analysis				
A. antiquata	4-10-14	9:30am	Mid	Sunny, no rain past 24 hours
E. denticulatum	4-10-14	1:45pm	High	Sunny, no rain past 24 hours
A. antiquata + E. denticulatum	4-11-14	4:00pm	Mid	Cloudy, constant rain day before
P. margaritifera	4-18-14	9:30am	Mid	Sunny, no rain past 24 hours
U. reticulata	4-18-14	11:15am	Low	Sunny, no rain past 24 hours
P. margaritifera + U. reticulata	4-21-14	9:30am	High-Mid	Sporadic, heavy rainfall for 2 days
Bacterial Analysis				
A. antiquata	4-16-14	10:45am	Low (spring)	Sunny, but rain in past 24hr
P. margaritifera	4-16-14	10:45am	Low (Spring)	Sunny, but rain in past 24hr
E. denticulatum	4-16-14	10:45am	Low (Spring)	Sunny, but rain in past 24hr

Table 1: The date, time, tide, and weather conditions during seawater and sand collection for all experimental set-ups

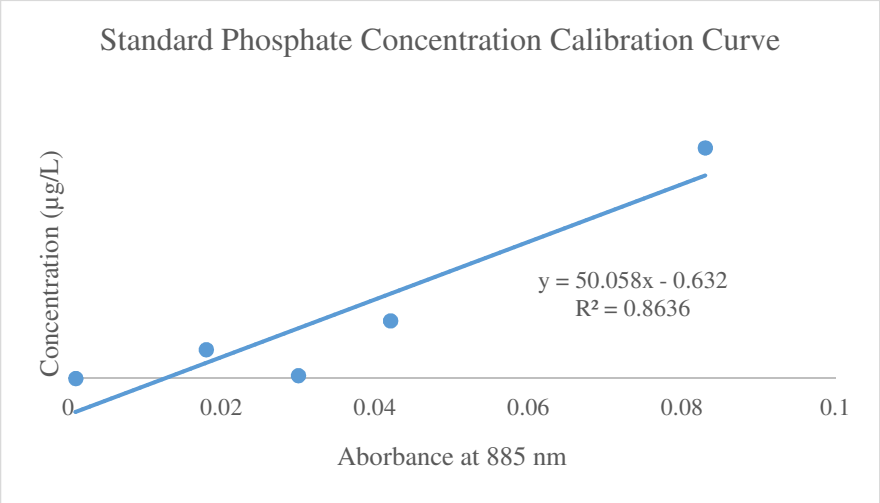
Experiment	pH Initial	pH Final	Average Temperature (˚C)	Length of Experiment
Nutrient Analysis				
A. antiquata	6	6	27.3	1 hour
E. denticulatum	6	6	30.4	3 hours
A. antiquata + E. denticulatum	7	7	28.2	3 hours
P. margaritifera	7	7	27.5	1 hour
U. reticulata	7	7	28	3 hours
P. margaritifera + U. reticulata	6	6	27.5	3 hours
Bacterial Analysis				
A. antiquata	7	7	28.4	2 hours
P. margaritifera	7	7	28.4	2 hours
E. denticulatum	7	7	28.4	2 hours

Table 2: The pH, average temperature (measured in 15 minute increments), and total time of experiment for all experimental set-ups in this study.

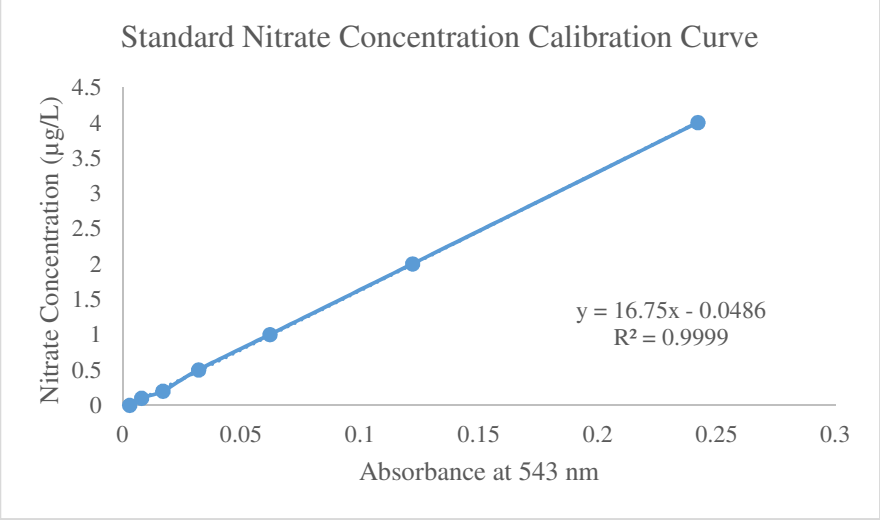
Experiment	Average Total Suspended Solids (g/350mL)	Two-tailed T-Test Results
A. antiquata	0.0267 ±0.0042	t= 2.7764, p= .0372
Control	0.0192 ±0.0006	
E. denticulatum	0.0318 ±0.0050	t= 2.7764, p= .0486
Control	0.0232 ±0.0018	
A. antiquata + E. denticulatum	0.0189 ±0.0004	t= 2.7764, p=. 0254
Control	0.0207 ±0.0008	
P. margaritifera	0.0210 ±0.0046	t= 2.7764, p= .0297
Control	0.0298 ±0.0003	
U. reticulata	0.0342 ±0.0041	t= 2.7764, p= .7225
Control	0.0385 ±0.0194	
P. margaritifera + U. reticulata	0.0295 ±0.0040	t= 2.7764, p=.1534
Control	0.0373 ±0.0066	

Table 3: The average results and standard deviation data for the Total Suspended Solids test of each experiment.

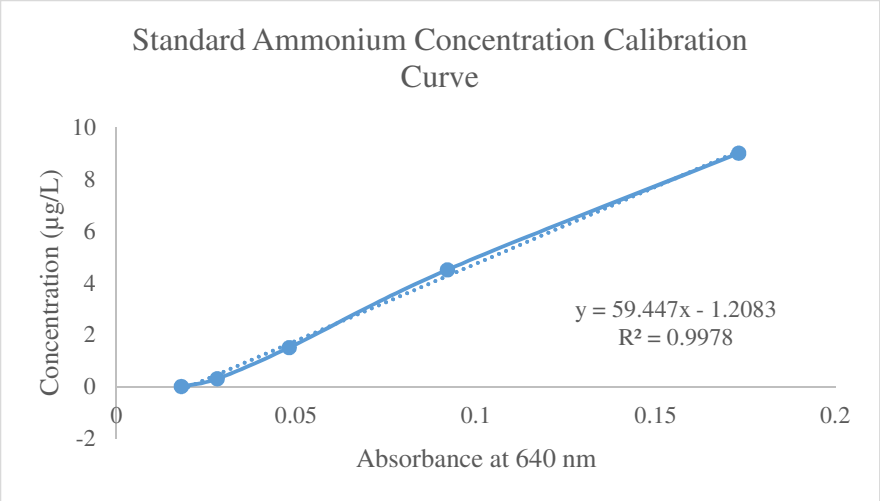
***Appendix II**: Nutrient Analysis Supplementary Materials*



Nitrate Concentration (µg/L)	Absorbance at 543nm
0	.003
.1	.008
.2	.017
.5	.032
1	.062
2	.122
4	.242



Ammonium Concentration (µg/L)	Absorbance at 640nm
.018	0
.028	.3
.048	1.5
.092	4.5
.173	9



Phosphate Concentration (µg/L)	Absorbance at 543nm
.001	0
.03	.05
.018	.5
.042	1
.083	4

Appendix IV: Miscellaneous

Agar Preparation:

M-Enterococcus Agar is used for the detection of fecal *Streptococcus* and *Enterococcus* groups
using the membrane filtration technique.

Composition (g/L):
Casein enzymic hydrolysate……..... 15.000
Papaic digest of soyabean meal …… 5.000
Yeast extract……. 5.000
Dextrose……. 2.000
Dipotassium phosphate……..... 4.000
Sodium azide…….. 0.400
2,3,5-Triphenyl tetrazolium chloride…. 0.100
Agar ……... 10.000
Final pH (at 25°C)= 7.2 ± 0.2

A ratio of 4.15 grams of agar base per 100mL of distilled water was suspended, and the mixture was heated to boiling to completely dissolve the medium. The agar was then immediately dispensed into petri dishes.

of agar base for *Enterococci* bacterial analysis.